Embryonic Development of the Testis

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Abstract

Testicular development is a very interesting aspect of male reproduction and fertility. The primordial germ cells migrate from yolk sac to the genital ridge along the wall of the hindgut and the dorsal mesentery to ultimately settle in the genital ridge that would give rise to testes. Sex-determining region of the Y chromosome (SRY) is the principal driver of testes development. Testes development culminates into descent of fully formed testes in the scrotum, which is necessary for facilitating spermatogenesis. Failure of testicular descent results in their retention in the inguinal canal or abdomen, often associated with azoospermia and infertility. This chapter provides a brief overview of the process of testicular development and descent with a glimpse of the consequences of the failure of testicular descent.

Keywords

Testis development • Germ cell migration • Testes descent • Cryptorchidism Azoospermia

Key Points

- The testes differentiate themselves earlier than the ovaries, namely, in the course of the 7th week.
- SRY gene on the Y chromosome is the primary driver for testis development, which in tum drives male sexual differentiation.
- Interestingly, the primordial germ cells migrate from yolk sac to the genital ridge along the wall of the hindgut and the dorsal mesentery.

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R. Singh, K. Singh (eds.), *Male Infertility: Understanding, Causes and Treatment*, DOI 10.1007/978-981-10-4017-7_2

- Migration of testis to the scrotum completes by the end of the 8th month, which is followed by small changes up to shortly after birth.
- Failure of testes descent can result in a number of abnormal positions of testes from inguinal canal to abdomen, called as cryptorchidism, which is often associated with azoospermia.

2.1 Introduction

Functional gonads are essential for sexual reproduction and the survival of higher animal species. The development of gonads is a particularly interesting event that is highly orchestrated in the form of origin, migration, and final settlement of the germ cells in the gonads. All these critical events take place in a tightly regulated temporal fashion. In humans, the sexual development and differentiation takes place at three levels: (1) chromosomal level, which is decided at the time of gamete fusion; (2) gonad level, which is the development of either testes or ovaries; and (3) physiological level, which is the development of secondary sexual organs and characters under the influence of hormones secreted by gonads. The primordial gonads are bipotential and have the capability to differentiate into ovaries or testis depending upon the molecular signals. The identification of the testis-determining gene, SRY, was a major discovery in the signals that set the path for development of either testis or ovaries. SRY is believed to be the master regulator of gonadal development, the absence of which results in ovarian development. Though a number of genes have been identified to be important for ovarian development, no master regulator of ovarian development has been identified.

In humans, the first important step of sexual differentiation takes place during the initial 7 weeks of the embryonic development that consists of several successive events starting with the establishment of genetic sex, development of the gonadal ridge, and immigration of primordial germ cells trailed by a sexually dimorphic differentiation of the gonadal anlagen into either testes or ovaries. Until this point of time, it is denoted as the indifferent stage of gonadal development, and no morphologically distinct sex differences can be observed in the developing human gonads. This developmental phase has a major influence on the later events of male as well as female paths since it establishes the hormonal dimorphism. This chapter details the differentiation of male gonads, covering the events from its first appearance through maturation to ultimate migration in the scrotum.

2.2 Overview of the Development of the Testes

The differentiation of the testes takes place more quickly than ovaries in the course of the 7th week (44 days). SRY is the principal driver gene for testicular differentiation. SRY initiates a series of gene expression, ultimately helping testes differentiation. The primordial germ cells are the bipotential cells that can give rise to both spermatozoa and oocytes. These are diploid like all other somatic cells and can

Table 2.1 Chronology of important early events in human male sex differentiation

Event	Age at start (dpc)	Size CRL (mm)
Genetic sex	0	
PGC migration from yolk sac	28	4
Formation of gonadal ridge	32	5
PGCs reach gonadal ridge	37	10
Male sex determination	42	15
Leydig cells appear	55	30
Androgen, INSL3 detectable	63	40
Testicular descent (first phase)	67	

dpc days post conception, *CRL* crown rump length ("sitting height")

already be found in human embryos in the primary ectoderm (epiblast) in the 2nd week of gestation. The first step in the organogenesis of testes is the differentiation of Sertoli's supporting cells (Karl and Capel 1998). The appearance of these cells in mice comes from the gonadal ridge, precisely the pluripotent coelomic epithelial cells (Table 2.1). The gene expression events triggered by the SRY result in the formation of intercellular membrane connections that would surround the primordial germ cells. With this, the gonadal cords start rising into the medulla. In males, cells of the mesonephric origin start accumulating on the outer side of gonadal cords and form peritubular myocytes. Gonadal cords develop into testicular cords and later into seminiferous tubules. The efferent ductules develop link with rete testis and the mesonephric duct. The influence of testosterone toward the end of the 8th week directs tight coiling of the cranial part of the mesonephric duct to develop into epididymis, while the exterior part of the duct remains as the deferens duct.

Post 8th week, certain mesenchymal cells in the testicular cord develop into Leydig cells, which further drive testosterone production. The mesenchyme between the testicular cords leads to the development of septa that divide each testis into lobules. The exact origin of these cells remains unknown. The mesenchyme at this stage also forms connective tissue layer between the testicular cords and the future tunica albuginea. The coelomic epithelium finally transforms into a mesothelium, just like other cavities. Finally, the testes are migrated to the scrotum. Migration of the testis apparently involves two phases; the initial stage is transabdominal migration, and the second stage is passage through the inguinal canal.

2.3 Formation of the Primitive Gonads

By day 32 post conception (pc), the gonadal anlagen can be recognized as combined bipotential structures in the developing human embryo. No morphological sexual dimorphism can be seen at this stage of development. Primordial germ cells (PGCs), which develop into gonocytes later on, cannot be observed at this time of gonadal development (Shawlot and Behringer 1995; Torres et al. 1995; Park and Jameson 2005).

The mesonephros at this stage also has primordium of the adrenal glands and the urinary system. The development of the urogenital system is regulated mainly by two transcriptional regulators: Wilms' tumor-associated gene 1 (WT1) (tumor suppressor) and steroidogenic factor-1 (SF1) (the orphan nuclear receptor). WT1 is a DNA- and RNA-binding protein with transcriptional and posttranscriptional regulation capacity. It is expressed in gonadal stromal, coelomic epithelial and immature Sertoli cells, interacts with the cAMP-responsive element-binding protein CITED 2, and is regulated by "paired box gene 2" (PAX2). SF1, expressed in the gonadal ridge, is a transcriptional regulator of steroid hydrolases, gonadotropins, and aromatase and is involved in the stabilization of intermediate mesoderm, follicle development, and ovulation. Furthermore, SF1 helps in regulating the anti-Müllerian hormone (AMH), dosage-sensitive sex reversal congenital adrenal hypoplasia critical region on the X chromosome protein 1 (DAX1), and steroidogenic acute regulatory protein (StAR). Normally these genes are expressed in the somatic testicular compartment and are important for normal testicular cord formation, and for the beginning of steroidogenesis they help in the differentiation of the Leydig and Sertoli cells. Sf1 knockout in mice results in the failure of gonadal and adrenal development, whereas the corresponding loss of function mutations in humans has a less prominent gonadal phenotype and adrenal insufficiency (Biason-Lauber and Schoenle 2000; Achermann et al. 2002; Park and Jameson 2005).

2.4 Cell Lineages

2.4.1 Primordial Germ Cells

By the end of the 5th week of conception, three different lineages of somatic cell types with bipotential fate dependent on their future paths constitute the gonadal anlagen. At this stage, immigrating primordial germ cells (PGCs) are colonizing the gonadal structures. After the final localization in the gonad, they are specified as gonocytes. The PGCs differentiate from epiblast-derived stem cells in the yolk sac. Expression of alkaline phosphatase, OCT3/4, and c-kit by the germ cells at this stage can be used to differentiate them from other cells. The PGCs migrate to the genital ridge under guidance of the extracellular matrix proteins expressed along the dorsal mesentery of the hindgut (Fig. 2.1). During the transit, the PGCs undergo active mitotic proliferation and expand in numbers by the time they reach gonadal anlagen. (Bendel-Stenzel et al. 1998; Wylie 1999). Gonocytes continue proliferation in the early testis shortly after determination and later become mitotically quiescent. The entry into meiosis is not allowed until much later in time. This decision is governed by the somatic cells since XY PGCs residing in an ovary follow the female path (McLaren and Southee 1997). Nevertheless, entry into meiosis may also be activated by mechanisms intrinsic to the germ cells (Morelli and Cohen 2005). Differentiation of the somatic cells (Leydig and Sertoli cells) in the male gonad continues even in

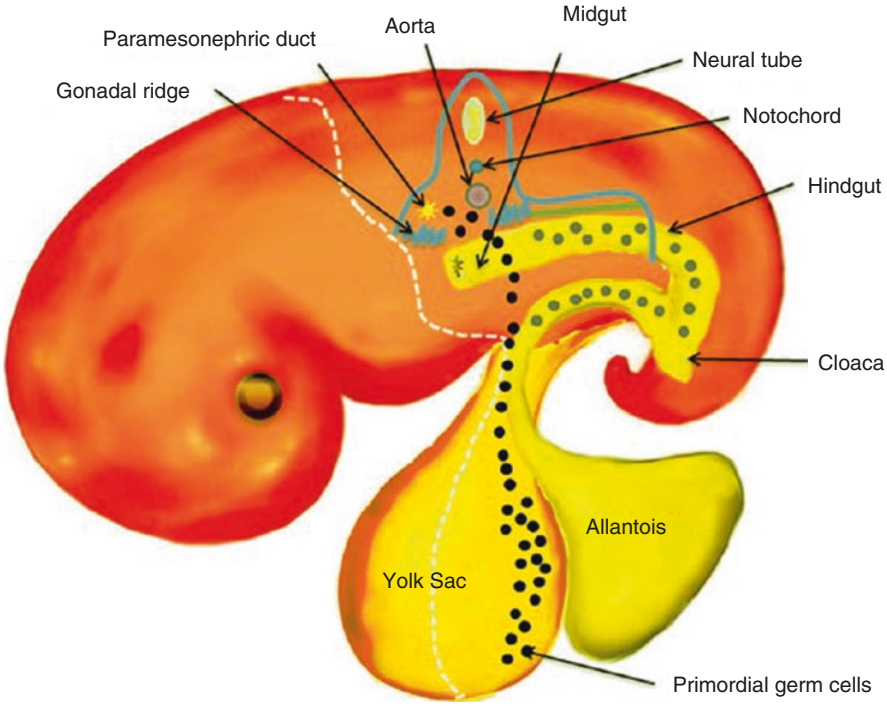

Fig. 2.1 Migration of the primordial germ cells from yolk sac to the genital ridge along the wall of hindgut and the dorsal mesentery

the absence of germ cells. This results in testosterone production that ensures pubertal development, but they are infertile due to Sertoli-cell-only syndrome (SCOS) (Söder 2007).

2.4.2 Somatic Cell Lineages in the Male Testis

Upon completion of the 6th week of embryonic development, four cell lineages consisting of Sertoli cells, Leydig cells, and peritubular cells and gonocytes can be identified in the indifferent gonad. The crucial somatic cell lineages are Sertoli cells, Leydig cells, and peritubular cells. Failure of differentiation and function of any of these lineages would result in severe phenotypes with respect to adult gonadal function and fertility.

2.4.2.1 Sertoli Cells

Sertoli cells are essential for testicular histogenesis and future functions. In adult testis, the Sertoli cells are nurse cells for spermatogenesis, creating niches for differentiation of spermatogonial stem cells and providing structural support, nutrients, and growth factors for the developing germ cells. Due to the fact that sperm

output in the adult testis is related to the number of Sertoli cells, the control of Sertoli cell proliferation in the developing testis is very important for future production of male germ cells (Petersen and Söder 2006). Pituitary follicle-stimulating hormone (FSH) and its receptor (FSHR) are important factors for Sertoli cell development. Any functional impairments of the FSHR may result in reduced fertility, a reduction of Sertoli cell numbers and, therefore, a reduction of testicular size combined with a reduction in circulating testosterone levels (Huhtaniemi et al. 1987; Simoni et al. 1997; O'Shaughnessy et al. 2006). Sertoli cell differentiation and proliferation is one of the most important steps in male sex determination. The fetal hypothalamic-pituitary-gonadal (HPG) axis is not yet operative, and FSH is not available during the first phase of Sertoli cell differentiation (Söder 2007). Therefore, Sertoli cell proliferation at this stage is controlled by other regulators (Petersen et al. 2001, 2002; Petersen and Söder 2006). A number of endocrine disruptors and inflammatory factors can disrupt Sertoli cell differentiation and proliferation at this stage (Petersen et al. 2002, 2004; Petersen and Söder 2006; Söder 2007).

Pre-Sertoli cells are first defined as cells of the supporting lineage expressing the sex-determining region on the Y chromosome (SRY). After SRY expression, the SRY-related HMG box 9 (SOX9), a gene with predominantly testis promoting activity, is expressed by the Sertoli cells and that leads to an upregulation of AMH, fibroblast growth factor 9 (FGF9), and prostaglandin D2 (PGD2). These genes affect the differentiation of the reproductive tract and therefore define male sex determination. This procedure is quickly trailed by morphological changes in the primitive gonad, therefore, embracing testicular elements such as the arrangement of testicular lines.

2.4.2.2 Leydig Cells

In the developing male, Leydig cells constitute another crucial testicular cell lineage. Leydig cells originate from steroidogenic precursor cells that migrate from the coelomic epithelium and mesonephric mesenchyme to colonize the indifferent gonad (Merchant-Larios and Moreno-Mendoza 1998; O'Shaughnessy et al. 2006; Söder 2007). These cells start to proliferate and differentiate at week 7 of human embryonic development under the influence of the Sertoli cell signals, such as AMH, desert hedgehog (DHH), and FGF9 (Clark et al. 2000; Colvin et al. 2001). The first generation of the Leydig cells is fetal type, which appears after the testes determination. Other Leydig cell types appear before puberty and after achieving puberty (Ge et al. 2006; Colvin et al. 2001). At the 8th week of human gestation, fetal-type Leydig cells start producing testosterone and other androgens (Svechnikov et al. 2010). Initially, they are regulated by the placental human chorionic gonadotropin (hCG), which shares on Leydig cells signaling receptors with pituitary LH, though the latter appears much later in the development when the HPG axis becomes established in the beginning of the second trimester of human pregnancy. At mid gestation, they constitute 40% of the total testicular cell mass. Leydig cells are situated in the interstitial compartment of the testis and increase their number during the first 2–3 months after birth (Svechnikov et al. 2010).

In addition to testosterone, a crucial hormone for differentiation of male external and internal genitalia, Leydig cells also produce SF1 that is necessary for steroidogenesis (Achermann et al. 2002) and insulin-like factor-3 (INSL3). INSL3 and its receptor RXFP2, together with androgens and AMH, are involved in the process of testicular descent. The first transabdominal phase of testicular descent occurs in human fetuses during weeks 8–16. Apart from its role in testicular descent, INSL3 seems to be an important paracrine mediator in male gonad and serves as a useful marker of Leydig cell differentiation (Ferlin et al. 2006).

In the coelomic epithelium, adrenocortical and gonadal steroidogenic cells share an embryonic origin and exist as one lineage before divergence into the gonadal and adrenocortical paths (Mesiano and Jaffe 1997). Additionally, the expression of adrenocorticotropic hormone (ACTH) receptor on fetal Leydig cells makes ACTH (ACTH) an important regulator of Leydig cell development. A common origin of testicular and adrenocortical tissue is also supported by abnormalities that affect both these organs together (Stikkelbroeck et al. 2001). In a similar way to Sertoli cells, Leydig cells represent an obvious target of disruptive actions of xenobiotics and EDCs (Söder 2007). In adult animals, these cells demonstrate a large regenerative capacity. Several growth factors have been implicated in Leydig cell regeneration and survival (Yan et al. 2000). Yet it is not yet clearly identified if this regeneration is driven by the resident Leydig precursor cells. A second possible hypothesis suggests that peritubular testis cells also represent a reserve pool of steroidogenic cells.

2.4.2.3 Peritubular Cells

Peritubular cells (PTCs) are required for early histogenesis of the seminiferous cords along the basal membrane of the seminiferous tubuli. Together with the basal membrane and the Sertoli cells, they form the blood-testis barrier and provide physical support for the Sertoli cells. In the postpubertal testis, they are supposed to add contractile forces, which are thought to be necessary for pushing tubular fluid and sperm release (Söder 2007). By the chemotactic signals received from the Sertoli cells, early PTCs and the cells contributing to the vasculature of the testis migrate from the adjacent mesonephros (Cupp et al. 2003). This migration process is a crucial step in sex determination and is SRY dependent. Normal SRY expression is related to GATA4, a gene also expressed by the PTCs. GATA4 also activates steroidogenic genes such as StAR, CYP11A, CYP17, CYP19, and HSD3B2, which are mainly expressed in the Leydig cells. Considering this and the fact that they are highly proliferative cells, PTCs demonstrate important features for normal testis development (Capel et al. 1999; Schmahl and Capel 2003), but their precise role in adult testicular function is still not known. Data accumulated lately indicate their possible role as a reserve or stem cell pool (Haider et al. 1995) and that they might be involved in the regeneration of Leydig cells after a disruptive injury.

2.5 Testicular Descent

Function of the postpubertal testes is dependent on their scrotal position. The procedure of testicular descent consists of two phases: the first transabdominal phase of descent followed by the inguino-scrotal phase aiming to transfer the testes to a scrotal position (Fig. 2.2). The first phase begins soon after testis determination and

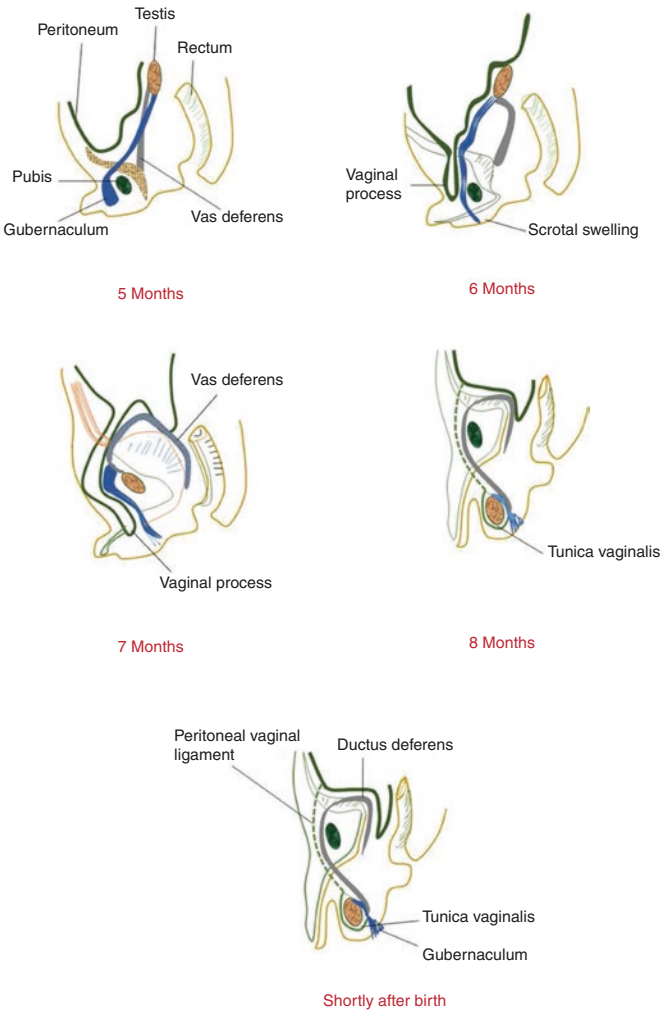

Fig. 2.2 The course of testicular descent: Between the 7th and the 12th week of gestation, the gubernaculum shortens and pulls the testes, the deferent duct, and its vessels onward. By the 6th month, the testes reach the orifice of the inguinal canal and cross it during the 7th month to reach their final position in the scrotum by the 8th month. After this, the inguinal canal contracts around the spermatic cord to complete the process. In the first year of life, the upper part of the vaginal process becomes obliterated, and peritoneo-vaginal ligament remains there. The lower portion persists as the tunica vaginalis testis

differentiation of Leydig cells and guides the testis from a position in the upper abdomen to the inner opening of the inguinal channel in the pelvic part of the abdomen. The testes with the epididymis and the proximal part of vas deferens finally move through the inguinal canal after week 18 of gestation. During the final 2 months of pregnancy, the testes usually take their scrotal position.

2.6 Perinatal Events in Testicular Maturation

During the third trimester of pregnancy, the fetal testes still produce large quantities of androgen but less than the peak activity at mid gestation. Although closer to birth, at term age, the hormonal activity of the testes declines, but it is still clearly measurable. However, soon after birth in both sexes of primates, but best recognized in human males, the first few months are a period of a very high hormonal activity of the testes and the hypothalamic-pituitary axis (Grumbach 2005). This period is often referred to as the mini-puberty and characterized by a hormonal surge of gonadotropins and testosterone. This is associated with proliferation of the Sertoli cells and some extent of germ cell development, i.e., transformation of gonocytes to Ad spermatogonia, at a time when gonadotropin, testosterone, and inhibin B reach high levels. More detailed studies have shown that LH value begins to increase 2 weeks after birth and decline to prepubertal values by 1 year of age in both sexes. FSH value also begins to increase 2 weeks after birth and decline to prepubertal levels by 1 year of age in boys and 2 years of age in girls. In parallel, testosterone level in boys often reach a peak of 10–15 nmol/L during the 2nd month of postnatal life but then decline to prepubertal low levels at 6 months of age (Forest 1975). The biological role of "mini-puberty" for future testicular and male reproductive function is unknown, but it has been speculated that it may play a role in the germ cell maturation and for the development of male gender identity.

2.7 Cryptorchidism: The Failure of Testicular Descent

Cryptorchidism or undescended testicle is a common developmental abnormality. Cryptorchidism is a stage in which testes fail to descend in the scrotal sac (Fig. 2.3). Since spermatogenesis takes place at a temperature 2–3 ° less than the body, failure of testicular descent leads to spermatogenic failure. The prevalence of cryptorchidism in the newborns is approximately 1–3%, but in premature children it increases to approximately 30% (Kolon et al. 2004). Cryptorchidism is of two types, unilateral and bilateral cryptorchidism. The prevalence of azoospermia in unilateral cryptorchidism is 13%, but this incidence increases up to 89% in bilateral cryptorchidism, suggesting that most of the cryptorchid individuals are azoospermic (Hadziselimovic and Herzog 2001). Bilateral cryptorchidism is more common as compared to unilateral cryptorchidism. The etiology of cryptorchidism is complex, involving a wide range of risk factors such as chromosomal, genetic and epigenetic alterations, hormonal imbalances, exposure to environmental toxicants, and the effect of endocrine disruptors.

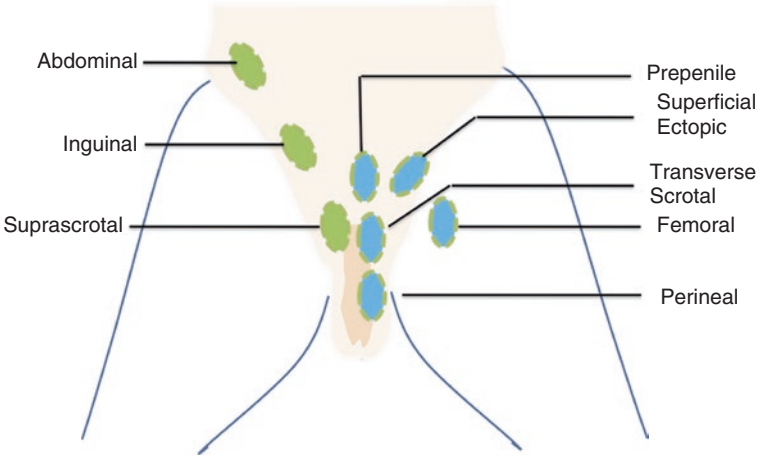

Fig. 2.3 Failure of testicular descent can take place at several levels, resulting in a variety of abnormal testicular positions from inguinal canal to abdominal. On the left side, normal testicular descent is shown (final scrotal position now shown), while the right side shows various positions of maldescent

2.8 Testicular Descent: Associated Disorders

Infertility is the primary disorder in cryptorchidism. Cryptorchidism is associated with spermatogenic alterations, which may range from normozoospermia to subfertility and azoospermia (Zimmermann et al. 1997, 1999). The severity of spermatogenic failure depends on the presence of unilateral or bilateral cryptorchid condition. Excryptorchid individuals may also display defects in spermatogenesis. A retrospective study described arthrogryposis multiplex congenita (AMC), a condition defined by the presence of multiple joint contractures at the time of birth to be associated with cryptorchidism (Fallat et al. 1992). The association of limb deformities was described by an external indirect compression of the inguino-scrotal region during the third trimester (Fallat et al. 1992).

Hypospadias, a congenital midline fusion defect of urethra leading to abnormal location of urethral meatus in males, is associated with increased risk of cryptorchidism (Tasian et al. 2010). Also, the incidence of hypospadias severity increased the risk of acquired cryptorchidism; however, the mechanism is still unexplained (Itesako et al. 2011). Further, the patients having disorder of sexual development (DSD) often have cryptorchid testis/gonads with ambiguous genitalia (Matsumoto et al. 2012). The risk of testicular cancer is 3–8 times high in cryptorchid individuals, and around 5–10% of patients with testicular cancer are excryptorchid (Whitaker 1988; Møller et al. 1996). It is thus an established risk factor for testicular germ cell tumor (TGCT). A recent study described that altered regulation of growth factor expression in the spermatogonial stem cell (SSC) somatic cell niche may impair the fine balance between SSC self-renewal and differentiation, which may drive the stem cells toward neoplastic transformation in cryptorchid individuals (Ferguson and Agoulnik 2015).

Acknowledgments The authors would like to thank the Council of Scientific and Industrial Research (CSIR), Govt. of India, for financial help under the network project, PROGRAM (BSC0101).

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